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Microvascular dysfunction following cardioplegic arrest and cardiopulmonary bypass

Jun Feng, Shawn Kant, Frank W. Sellke

Department of Surgery, Rhode Island Hospital, Providence, RI 02903, USA.

Correspondence to: Dr. Jun Feng, Surgery, Rhode Island Hospital, 1 Hoppin Street, Coro West Room 5229, Providence, RI 02903, USA. E-mail: jfeng@lifespan.org

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Abstract

Cardioplegia and cardiopulmonary bypass (CP/CPB) during cardiac surgery may cause systemic microvascular dysfunction. CP/CPB is associated with significant alterations in myogenic tone, agonist-induced vasomotor response, and endothelial function in various organs and vascular beds. These alterations can result in vessel spasm, organ malperfusion, and tissue damage. This review summarizes the current state of research in this field.

Keywords: Microcirculation, microvascular function, cardiac surgery, cardioplegia, cardiopulmonary bypass, vasomotor tone, myogenic tone, endothelial function

INTRODUCTION

During cardiovascular surgery, cardioplegia (CP) and cardiopulmonary bypass (CPB) may influence *in vivo* vasomotor control and microvascular function, and as a result, affect organ perfusion. In general, basal perfusion increases immediately following surgery while the hyperemic response decreases. The diminished reactive hyperemic response has been hypothesized to be due to coronary vasodilatation that occurs due to tissue ischemia after surgery. In a study by Hiratzka *et al.*[1], the peak-to-resting flow velocity ratio following a coronary occlusion diminished from 4.4 ± 0.2 before bypass to 3.0 ± 0.3 after bypass. In a canine model reported in the same study, similar findings were apparent. During this same period, left ventricular perfusion increased, mean arterial pressure and coronary vascular resistance decreased, and myocardial oxygen consumption was unchanged.

These clinical and experimental studies suggest that major coronary vasodilatation occurring in the early period following CP/CPB when these modalities are used. In another study by De Backer *et al.*[2], hemodynamic and microcirculatory variables were examined before and after cardiac surgery with or without CPB. Vascular function was also examined in a non-cardiac surgical control group. Within hours of cardiac surgery, reductions in microvascular perfusion were more severe in patients undergoing CPB than in off-pump patients. Meanwhile, alterations in vascular function had normalized in the non-cardiac surgical controls at this time. In patients undergoing either on pump or off pump cardiac surgery, the microcirculatory alterations (i.e., extent of impaired microvascular perfusion and density of perfused vessels) decreased with time but persisted for more than 24 h after completion of the operation.

Interestingly, the degree of microvascular changes appeared to be related to tissue ischemia and correlated with the peak lactate levels after surgery. Myocardial blood flow and blood flow through other organs are critically important aspects of physiology to prevent ischemic injury. These are most accurately assessed with direct *in vivo* measurement. However, *in vitro* microvascular studies may allow us to better examine changes in specific vasomotor pathways and mechanisms compared to the *in vivo* assessment of organ perfusion. For example, when atrial and skeletal muscle arterioles harvested from patients were examined, the contractile response elicited by vasopressin in atrial coronary vessels was increased after surgery[3]. In contrast, the responses to norepinephrine, endothelin-1, and thromboxane A2 (TXA2) were decreased[4-7]. This demonstrates a differential effect of cardiovascular surgery on vascular reactivity depending on the agonist examined and even which vascular bed is examined. Thus, not only are *in vitro* vascular responses markedly abnormal after CP/CPB, but there are clinical *in vivo* correlates of these changes as well. Other studies also demonstrate potentially clinically important findings in the study of the microcirculation after cardiovascular surgery, namely disturbances in microcirculatory perfusion and adverse clinical outcomes[8,9].

This review will explore three different domains of microvascular dysfunction during and after CP/CPB, summarizing the current state of research and considering open possibilities for future investigations. The focus will be on post-CP/CPB alterations in myogenic tone, agonist-induced vasomotor tone, and endothelial dysfunction in the coronary and peripheral microvasculature.

MYOGENIC TONE AND CARDIOPLEGIA/CARDIOPULMONARY BYPASS

Background

Vascular tone is determined by many separate factors present in the various component of the blood vessel. These include the endothelium, vascular smooth muscle, surrounding tissue such as myocardium in the case of the heart, and neurohumoral factors. One factor affecting tissue perfusion is myogenic tone, critical for autoregulatory control of perfusion. Myogenic tone is an intrinsic property of vascular smooth muscle in many (but not all) vascular beds and plays an important role in regulation of the coronary and peripheral microcirculation (note that certain vascular beds, such as adipose arterioles, do not exhibit myogenic tone). Myogenic tone provides a baseline level of spontaneous vascular wall tone, allowing the microvasculature to respond to changes in blood flow and pressure. It also provides a baseline tonic level of vascular tone against which vasoactive mediators can modify vessel diameter. Changes in blood flow produce corresponding changes in microvascular resistance that are controlled by the contraction or relaxation of the arteriolar smooth muscle cells, with resulting changes in vessel diameter. Thus, myogenic tone often manifests as contraction of a vessel after a sustained increase in blood pressure triggers enough vasodilation to reach a critical point, after which vessel wall tension and transmural pressure respond with an elastic rebound effect. A key purpose of myogenic tone is likely to prevent unchecked passive dilation of vessels in response to increased intraluminal pressure[10].

Several mechanisms exist that regulate vascular myogenic tone. Ion channels such as TRPC6 and TRPM4 help mediate pressure-induced vascular smooth muscle depolarization[11]. Protein kinase C (PKC) serves as a regulator of the TRPM4 channel, and studies testing PKC inhibition have revealed attenuated pressure-induced myogenic vasoconstriction in human cerebral arteries[12]. PKC itself is sensitive to levels of intracellular calcium, with increases in intracellular calcium triggering heightened activation of PKC. Indeed, influx of calcium through voltage gated calcium channels has been shown to induce myogenic vasoconstriction in *in vitro* patch clamp experiments and intact vessel preparations[13,14]. Most myogenic responses are related to calcium influx that is thought to occur through L-type voltage gated calcium channels; however, T-type voltage gated calcium channels may also influence myogenic autoregulation in various vascular beds, as seen in animal models[15].

Epidermal growth factor receptor tyrosine kinase (EGFRtk) has been shown to affect coronary arteriole myogenic tone in coronary arterioles isolated from mouse models[16]. Intramural pressure leads to metalloproteinase 2/9 activation, release of heparin-binding EGF, and transactivation of EGFRtk[17]. Activation of EGFRtk by microvascular intraluminal pressure initiates a downstream intracellular signaling cascade involving JAK, STAT, Akt, and several other second messenger/effector molecules that ultimately results in enhanced myogenic tone/vasoconstriction. Likewise, the ERK1/2-JAK-STAT3 intracellular complexes have been implicated in myogenic tone of coronary arterioles[16].

Other studies implicate structural molecular components of vascular smooth muscle cells, including actin polymerization and cytoskeletal stabilization[18], and integrins[19] in the myogenic vascular responsiveness. Integrins specifically appear to be implicated in tyrosine phosphorylation cascades and mitogen activated protein kinase (MAPK) action, both of which are required for myogenic tone.

The effect of cardioplegia and cardiopulmonary bypass on myogenic tone

CP/CPB are associated with reduced coronary and peripheral microvascular myogenic tone across animal models and in patients[14,20-26]. These changes often manifest as post-CP/CPB systemic hypotension and in the extreme case may result in poor organ perfusion and injury. Several possible mechanisms have been put forward to explain this phenomenon. For example, blood cardioplegia and CPB reduces levels of ERK1/2 activation in coronary arterioles, which were correlated with reductions in myogenic tone before and after CP/CPB[22]. ERK1/2 regulates coronary vascular smooth muscle contraction, and pharmacologic inhibition of MEK1/2, a kinase upstream of ERK1/2, also results in diminished myogenic tone. PKC also influences ERK1/2 activation, suggesting that similar signal transduction cascades may be at play, affecting coronary myogenic tone[27,28].

Voltage-gated calcium channel function also appears altered in the microvasculature following CP/CPB. In human skeletal muscle arterioles, post-CPB myogenic tone has been associated with increased large conductance (BK_{Ca}) calcium-activated potassium channel activity[23]. Treatment with BK_{Ca} channel antagonist iberiotoxin significantly increased post-CPB microvascular myogenic tone; however, intermediate, and small conductance K_{Ca} channels appeared to have minimal effect on myogenic tone[23]. One result of protein kinase activation in vascular smooth muscle cells may be modulation of BK_{Ca} channel activity by phosphorylation of the channels themselves or assorted regulatory proteins[29]. Curiously, protein expression levels of BK_{Ca} channel were not altered in the microvascular smooth muscle following CPB, suggesting that posttranslational modifications to the channel (e.g., affecting open probability or duration) or alterations in downstream signaling are likely the driving force behind observed effects[23].

Either normothermic or hypothermic blood cardioplegia has been proposed as a potentially superior protocol to classic hypothermic crystalloid cardioplegia in preventing microvascular dysfunction and promoting myocardial protection during CP/CPB[10,30]. The rationale is that cold blood more closely approximates normal physiology and may provide more buffering capacity than cold crystalloid solution. However, debate continues concerning this topic, and little actual data conclusively demonstrates a clear clinical benefit of blood over crystalloid cardioplegia. In a porcine model of CPB comparing intermittent cold *vs.* continuous warm blood cardioplegia, both protocols of cardioplegia produced similar reductions in microvascular myogenic tone, suggesting that warm blood and intermittent cold cardioplegia have minimally different effects in that aspect[24]. However, other studies involving porcine models of hyperkalemic crystalloid cardioplegic solution *vs.* blood cardioplegia suggest that blood cardioplegia decreases myogenic tone to a lesser degree than cold crystalloid solution[25]. Clearly more studies, especially clinical studies involving patients, are needed before any definitive conclusions can be drawn.

Remaining questions

Several clinical variables that can potentially affect post-CPB changes in myogenic tone remain uninvestigated. For example, preoperative conditions such as diabetes may affect the outcomes of cardiovascular surgery. This is important because diabetes is a condition present in many patients undergoing coronary artery bypass grafting under CPB due to the high prevalence of coronary artery disease among this patient population[31]. While diabetes appears to alter myogenic tone in the microvasculature in humans post-CP/CPB, the specific mechanisms that drive these changes remain unclear[31]. Likewise, hypertension is another condition prevalent in many patients with cardiovascular disease. Hypertension may increase baseline myogenic tone and alter the structure and function of large arteries[32]; however, the specific contribution of preoperative hypertension to post-CPB myogenic tone remains to be elucidated. A similar lack of literature exists for the roles of age and gender.

AGONIST-INDUCED VASOMOTOR TONE AND CARDIOPLEGIA/CARDIOPULMONARY BYPASS

Agonist-induced vasomotor tone of the microvasculature refers to the contribution of neurotransmitters from the autonomic nervous system, and other vasoactive factors (e.g., hormones and neuropeptides), to the degree of tension within the vascular smooth muscle of arteries and arterioles. As with myogenic tone, CP/CPB has been shown to alter microvascular agonist-induced vasomotor tone, potentially contributing to observed postoperative microvascular dysfunction such as postoperative vasoplegia, hypotension, and organ malperfusion[10]. Dysfunctional agonist-induced vasomotor tone manifests differently in distinct vascular beds, presumably due to differences in receptor expression and other more distal mechanisms; specific details of any potential mechanisms remain to be elucidated. For example, consider decreased microvascular resistance in the peripheral and skeletal circulation versus increased propensity to spasm in the coronary, pulmonary, mesenteric, and cerebral circulations [Table 1][10,31].

Autonomic nervous system: α- and β-adrenergic receptors, phenylephrine

The autonomic nervous system contribution to vasomotor tone relies in large part on the sympathetic nervous system, which controls vascular diameter through norepinephrine action and epinephrine on α- and β-adrenergic receptors located on vascular smooth muscle cells. α-adrenergic receptors are important vasomotor mediators of blood vessels across the body[33]. α-1 receptors operate through a G-protein coupled receptor (GPCR) mediated pathway involving phospholipase C, elevated intracellular calcium levels, and activation of PKC, all of which lead to smooth muscle contraction and vasoconstriction. α-2 receptors have more mixed effects and operate through a GPCR pathway that downregulates adenylyl cyclase, reducing levels of cyclic adenosine monophosphate (cAMP). Curiously, following CP/CPB, α adrenergic receptors do

Table 1. Vasomotor agonist responsiveness following cardioplegic and cardiopulmonary bypass (CBP/CP)

Agonist	Vasomodulatory effect	CPB/CP induced changes	Ref.
α-adrenergic agonist phenylephrine	Vasoconstriction	Diminished responsiveness in human coronary arterioles	[4,6]
β-adrenergic agonists	Vasodilation	Decreased responsiveness in human skeletal muscle arterioles	[36]
Neuropeptide Y	Coronary artery vasoconstriction and myocardial contraction	No changes in responsiveness; gender-based changes in human atrial tissue neuropeptide Y expression levels	[40,42]
Endothelin-1	ETA receptor: vasoconstriction ETB2 receptor: nitric-oxide mediated vasodilation	Increased plasma levels of endothelin-1, immediate postoperative period Decreased responsiveness of human coronary and skeletal muscle arterioles	[5,43-47]
Serotonin	5HT1B, 2A, and D receptors: vasoconstriction 5HT2B and 7 receptors: vasodilation	Impaired 5HT1B receptor mediated contractile response in skeletal vasculature Increased coronary and pulmonary vasoconstriction	[48-54]
Vasopressin	Vasoconstriction	Increased responsiveness in human coronary arterioles Decreased responsiveness in human skeletal muscle arterioles	[3]
Thromboxane A2	Vasoconstriction	Reduced responsiveness in human coronary arterioles	[7,54]

not show protein-level changes in expression[34]. However, experiments also show that post-CPB response to phenylephrine, an α-1 adrenergic agonist, is markedly diminished in human coronary arterioles[4]. Hence post-translational modification to receptors, alterations in receptor trafficking (resulting in fewer membrane bound receptors, even with an unchanged amount of total receptors) or changes in downstream signaling are likely mediating this altered α adrenergic response. This may be the result of receptor desensitization or impairment of the cellular pathway responsible for the contractile action due to activation of the α-1 adrenergic receptor.

β-adrenergic receptors are important vasodilators in the microvasculature of the myocardium, skeletal muscle and other tissues, acting through a GPCR mediated intracellular signal transduction cascade[35]. Elevated levels of cAMP result from β-2 receptor stimulation; this activates protein kinase A (PKA), which phosphorylates myosin light chain kinase thereby triggering smooth muscle relaxation. *In vitro* experiments on microvessels removed from human skeletal muscle show decreased responsiveness to β-2 receptor agonists following cardiopulmonary bypass, suggesting a postoperative deficit in β-2 receptor mediated vasodilation[36]. Given that β-2 receptor expression levels were unchanged, these changes are most likely mediated by changes to downstream elements of the β-2 receptor transduction cascade[36].

Neuropeptide Y

Neuropeptide Y is widely distributed in the central and peripheral nervous system, and often functions as a sympathetic co-stimulator alongside norepinephrine[37]. Neuropeptide Y is also abundant in the heart and coronary arteries, facilitating coronary artery constriction and myocardial contraction[38]. Of the many different neuropeptide Y receptors currently identified, the Y1, Y2, and Y5 receptors appear to be the most important regulators of cardiovascular homeostasis[38,39]. Each of these receptors function through a GPCR signal transduction cascade culminating with inhibition of adenylyl cyclase and reduced action of PKA. The Y1 receptor specifically may also act through a phospholipase C-dependent pathway to stimulate vasoconstriction via changes in intracellular calcium levels. Human skeletal muscle microvasculature has been shown to respond to neuropeptide Y agonists with dose-dependent manner vasoconstriction, raising the possibility that aberrations in neuropeptide Y could influence vasomotor dysfunction in the peripheral vasculature following CPB[40]. CP/CPB significantly altered the gene/protein expression of neuropeptide Y in human blood and myocardium[41]. Expression of neuropeptide Y in human atrial tissue following CPB appears to show a level of gender stratification: samples from men exhibited reduced post-CPB neuropeptide Y mRNA while samples from women exhibited increased neuropeptide Y mRNA[42].

However, there appears to be no change in the response of human skeletal microvessels to neuropeptide Y following CPB, along with no changes in Y1 receptor abundance in the microvessels[40]. Additional studies will be required to verify these results.

Endothelin 1

Endothelin-1 (ET-1) is an important vasomodulator secreted by endothelial cells[43,44]. ET-1 functions through two different types of GPCRs: ETA and ETB receptors. ETA and ETB2 receptors are found in vascular smooth muscle and trigger vasoconstriction, along with cell proliferation (ETA) and inflammation (ETA)[44]. Conversely, the ETB2 receptor is predominantly on endothelial cells and functions in nitric oxide mediated vasodilation[44]. Following CPB during coronary artery bypass grafting, plasma endothelin-1 levels in systemic arterial and pulmonary circulations increased between 50%-85% in the immediate postoperative period[45,46]. However, *in vitro* contractile response of human coronary and skeletal muscle arterioles to ET-1 was shown to be markedly decreased following cardiopulmonary bypass[5,47]. Molecular analysis revealed no significant changes in ET receptor expression levels, thereby implicating posttranslational modifications, dysregulated receptor trafficking, or downstream signaling effects as potential mechanisms of ET-1 driven vasomotor dysfunction via ETA receptors and PKC-α signaling pathways[5,47].

Serotonin

Serotonin, released in high levels during platelet activation, functions as an important vasomodulator in the circulation through action of various serotonin receptors (5HTRs). During acute coronary syndromes, serotonin released from platelets may cause further platelet aggregation and affect downstream vasomotor tone. Serotonin may promote vasoconstriction predominantly by means of the 5HT2A receptor, with contributions from the 5HT1B and D receptors[48]. Meanwhile, certain vessels such as human umbilical arteries and skeletal muscle arterioles have been shown to dilate in response to stimulation of other serotonin receptors, such as the 5HT2B and 5HT7 receptors[48]. In addition to vasomodulatory effects, serotonin is also capable of activating phospholipase A2 (PLA2), the key initial enzyme involved in arachidonic acid metabolism and generation of inflammatory mediators like prostaglandins, lipoxygenases, and thromboxane A2 (TXA2)[49,50]. Most of the serotonin receptors belong to the GPCR family.

CP/CPB impairs the 5HT1B receptor mediated contractile response to serotonin postoperatively in the skeletal vasculature[51]. However, there appear to be no changes in the expression levels of skeletal vascular 5HT1B receptors following CPB, indicating that the observed effects are likely due to posttranslational or downstream signaling modifications[51]. In contrast, serotonin-induced coronary and pulmonary vascular contraction have been shown to increase following cardioplegia-reperfusion and are associated with increased expression of the pro-inflammatory enzymes, PLA2 and cyclooxygenase 2 (COX2), key mediators of prostaglandin, thromboxane, and prostacyclin synthesis[52-55]. Furthermore, 5HT1B receptor *mRNA* expression increased following CPB in coronary arterial smooth muscle, suggesting that increased 5HT1B receptor expression may mediate a hypercontractile state in the coronary microcirculation following CPB.

Vasopressin

Vasopressin is released from the posterior pituitary gland during stress such as myocardial infarction and cardiogenic shock. In addition, vasopressin has long been used as a drug for maintaining systemic blood pressure and minimizing hypotension after cardiac surgery[56]. As the name suggests, vasopressin induces constriction of arterial vascular smooth muscle by binding to V1 receptors[57]. V1 receptors primarily function through GPCR pathways, inducing activation of PLA2, phospholipase C, and calcium influx, the latter being most relevant to vasoconstriction. Circulating vasopressin increase in a variety of pathologic states including hypotension, hemorrhagic shock, and hypoxia[3]. Experiments with human coronary arterioles revealed increased responses to vasopressin following CP/CPB, associated with increased

postoperative expression of V1 receptors[3]. Curiously, human skeletal muscle arterioles exhibited decreased contractile response to vasopressin following CP/CPB[3]. Future studies are required to elucidate the specific mechanisms of altered vascular responsiveness to vasopressin in the coronary and peripheral microcirculations, although observed aberrations in PKC and MAPK systems (implicated in V1 receptor signal transduction), along with increased oxidative stress following CPB, may be at play[22,58,59].

Thromboxane A2

TXA2 is a pro-inflammatory, prothrombotic product of cyclooxygenase 1 and thromboxane synthase action on arachidonic acid and prostaglandin H-2[60]. With respect to vasomotor tone, TXA2 acts on thromboxane receptors of vascular smooth muscle, inducing activation of phospholipase C, release of intracellular calcium stores into the cytosol, and activation of myosin light chain kinase[60]. CPB induces a generalized systemic inflammatory state that contributes to vasomotor dysfunction, involving elevated levels of PLA2, TXA2, and thromboxane synthase, postoperatively[10,52,54]. High postoperative TXA2 may be responsible for heightened platelet activation and an overall prothrombotic state following CPB, a supposition reinforced by the use of thromboxane receptor antagonists and thromboxane synthase inhibitors to reduce post-CPB thrombosis[7]. However, despite higher TXA2 levels, the post-CPB contractile response of human coronary arterioles to TXA2 analogs is markedly impaired postoperatively[7]. It is possible that sustained high levels of TXA2 may induce decreased sensitivity to TXA2-mediated vascular smooth muscle constriction. Other elements of the post-CPB inflammatory response such as nitric oxide, free radicals, and activated neutrophils may also contribute to increased vascular permeability and diminished responsiveness to TXA2[7].

Further considerations and future directions

As with myogenic tone, several additional clinical variables can influence the effects of CP/CPB on agonist-induced vasomotor tone. Among patients with diabetes, post-CP/CPB attenuation of phenylephrine induced coronary arteriolar vasoconstriction, serotonin induced peripheral microvascular constriction, ET-1 induced peripheral vasoconstriction, and coronary nitric-oxide mediated vasodilation are more pronounced compared to the responses observed in non-diabetic patients[4,31,51]. Meanwhile, the coronary arteriolar contractile response to vasopressin exhibits more profound increases following CP/CPB in diabetic *vs*. nondiabetic patients[3]. Furthermore, studies are needed to examine the effect of other pervasive preexisting conditions, such as hypertension, hyperlipidemia, and clinical variables such as sex and age, on agonist-induced vasomotor tone following CP/CPB.

ENDOTHELIAL DYSFUNCTION AND CARDIOPLEGIA/CARDIOPULMONARY BYPASS

Endothelial dysfunction is a broad term referring to: (1) immune response induced alterations to the structure and permeability of the vascular endothelium; and (2) disturbances in the relative production/availability of nitric oxide, and other endothelium-derived relaxing and contracting factors[61]. During cardioplegia, administration of cardioplegic solution (often cold hyperkalemic solution) arrests myocardial contractility to facilitate open heart surgery, after which heart function is restored through reperfusion[62]. This process, and the accompanying ischemia-reperfusion, can damage the coronary endothelium that may have detrimental effects on postoperative cardiac function[59,63-69]. Moreover, endothelial injury extends far beyond the coronary circulation alone; the vascular beds of many organ systems, including the pulmonary and mesenteric circulations, sustain damage following CP/CPB[70-72].

Endothelial derived relaxing factor: nitric oxide

The vascular endothelium produces a vast array of vasoconstrictive and vasodilatory substances that influence vascular tone, immune cell/platelet-vessel wall interactions, and the overall health of vascular smooth muscle[73]. An important endothelium-derived vasodilatory substance is so-called endothelial-

derived relaxing factor, which in arteries, veins and cultured vascular endothelial cells has been identified as nitric oxide. Nitric oxide functions as an important regulator of blood flow and tissue oxygenation by regulating vasomotor tone of vascular smooth muscle. In addition, nitric oxide inhibits mitochondrial reactive oxygen species production, inhibits inflammation, and quenches superoxide - thereby removing its own presence and creating peroxynitrite in its place. Normal, healthy endothelial cells exhibit constitutive activation of endothelial nitric oxide synthase (eNOS); activated endothelial cells, inflammatory cells, cardiomyocytes, and vascular smooth muscle cells express inducible nitric oxide synthase (iNOS)[10].

Both eNOS and iNOS produce nitric oxide as a byproduct of the conversion of L-arginine to L-citrulline. A gas, nitric oxide activates soluble guanylyl cyclase in the vascular smooth muscle, which in turn converts guanosine monophosphate to cyclic guanosine monophosphate, leading to activation of protein kinase G and phosphorylation of calcium channels[74]. This series of events results in vasodilation. Beyond vasomotor effects, nitric oxide also inhibits leukocyte adhesion and platelet activation[10]. Reduced activity of eNOS has been observed following CP/CPB, mediated by a variety of potential mechanisms including depletion of L-arginine, changes in intracellular calcium concentrations, and cell membrane injuries[59,75-79]. Generation of free radicals during reperfusion after cardioplegia leads to increased nitric oxide breakdown[80]. In addition, endothelial exposure to byproducts and mediators of inflammation, including activated complement and neutrophils, further attenuates the ability of vascular endothelium to generate nitric oxide following CP/CPB[81,82].

PGI2 and COX

Prostacyclin (PGI2) is an important vasodilator synthesized in vascular endothelium[50]. It also functions as a counterweight to the pro-thrombotic, vasoconstrictive effects of TXA2[50]. PGI2 is synthesized from prostaglandin H2 (PGH2) with the assistance of PGI synthase. PGH2 is itself a product of arachidonic acid metabolism, the result of cyclooxygenase (COX) enzyme action[50]. Two COX isoforms have been identified in human cells: COX1 and COX2. COX1 is a housekeeping isoform constitutively expressed in most cells of the body; *COX2* expression is induced by inflammatory cytokines in immune cells and activated endothelial cells[83]. Studies have shown that enhanced activity of COX2 leads to a shift in favor of PGI2 over TXA2, while reduced COX2 activity (or predominant COX1 activity) produces the opposite effect[84-86]. Inflammatory cytokines and endothelial stress have been demonstrated to upregulate COX2 in *in vitro* models[50].

COX2 expression increases during reperfusion following CP/CPB in a variety of vascular beds, including coronary, cerebral, and pulmonary circulation. This observed effect is consistent with the principle of inflammation-induced changes in vascular endothelial cells[87,88]. Meanwhile, COX1 levels remain unchanged. Other experiments using skeletal muscle microvessels confirmed COX2 upregulation post-CPB *vs*. pre-CPB, and associate this with altered peripheral microvascular reactivity (e.g., enhanced bradykinin-induced relaxation responses)[89]. Despite enhanced PGI2 levels due to enhanced COX2 action, the overall effect of higher endothelial *COX2* expression following CP/CPB appears to be more potent vasoconstriction, given how administration of COX2 inhibitors attenuates vasoconstriction and release of contractile prostanoids following CP/CPB[88].

Endothelium-derived hyperpolarizing factor

Endothelium-derived hyperpolarizing factor (EDHF) essentially refers to an endothelium-dependent pathway involving calcium, opening of specific potassium channels (e.g., the calcium-sensitive potassium channels such as $K_{Ca}2.3$ and $K_{Ca}3.1$), and vascular endothelial and smooth muscle hyperpolarization[90-93]. Such calcium-activated potassium channel (K_{Ca}) pathways are thought to play important roles in dilation of coronary arteries, and animal models have implicated bradykinin in activation of coronary artery K_{Ca}

channels[66,94]. Even in peripheral arteries, intermediate and small conductance K_{Ca} channels have been demonstrated as mediators of vasodilation[95].

CP/CPB appears to induce changes in coronary and peripheral EDHF pathways/responsiveness. Studies on human atrial and coronary microvessels reveal significantly reduced post-reperfusion relaxation responses after activation of intermediate and small conductance K_{Ca} channels following CPB, despite no alterations in channel polypeptide or *mRNA* expression levels[96-98]. A similar situation of reduced responsiveness to K_{Ca} channel activation has been demonstrated in human skeletal muscle arterioles following CPB[95]. Pharmacologic intervention in the EDHF pathway has shown promise for mitigating endothelial dysfunction following CP/CPB. For example, in animal and human coronary artery endothelial cells, administration of a small conductance calcium-activated potassium channel activator before and during cardioplegic hypoxia significantly improved overall coronary endothelial function, assessed by preserved responsiveness to bradykinin, ADP, and substance P[98-100].

Immune activation and oxidative stress

Activation of the complement system and coagulation cascades occur when blood encounters the CPB circuit[101,102]. Complement factors (e.g., anaphylaxins C3a and C5a) can induce synthesis of pro-inflammatory cytokines, like IL-1, IL-6, and TNF-α which in turn stimulate endothelial changes that facilitate neutrophil chemotaxis, extravasation, and tissue edema. Activation of the classical complement pathway specifically may cause direct vascular endothelial injury[103]. The terminal membrane attack complex formed from complement fragments C5b-9 has also been shown to cause direct endothelial injury in porcine coronary and mesenteric arteries following ischemia-reperfusion and CPB[82,104]. Administration of anti-C5a antibodies before onset of CPB in porcine model exhibits reduced coronary/pulmonary endothelial dysfunction assessed through degree of impairment of endothelial relaxation to various agonists (e.g., clonidine)[105,106].

Leukocyte activation may also damage vascular endothelium during and after CPB through production of reactive oxygen species, proteolytic enzymes, and inflammatory cytokines[10,107]. Increased expression of endothelial selectins after onset of CPB allows for increased neutrophil tissue infiltration across vascular walls. Further evidence for a role for leukocytes in post-CPB endothelial dysfunction comes from use of leukocyte-depleted (or inhibited) blood for reperfusion following cardioplegia in pig models, which demonstrated improved postoperative myocardial perfusion, along with improved coronary and cerebral vascular function[107-109]. Finally, in porcine model of CPB, addition of superoxide dismutase significantly preserved the vasomotor responses of coronary microvessels to serotonin and ADP, suggesting a role for oxygen derived free radicals in endothelial dysfunction[59,63].

Endothelial barrier integrity

CPB reduces the integrity of endothelial barrier function by compromising endothelial adherens junctions, leading to increased vascular permeability[31,110]. Animal and human studies demonstrate that CP/CPB is associated with increases in VEGF and/or its receptors in blood and tissues and enhanced tissue edema[111-113]. In porcine myocardial tissue following CPB, levels of small molecular weight fragments of VE cadherin, β-catenin, and gamma-catenin increased relative to pre-CPB levels, indicating enhanced protein degradation[114,115].

Experiments in human skeletal muscle arterioles and right atrial tissue report increased phosphorylation of vascular endothelial cadherins, along with overall decreases β-catenin levels[116,117]. Thus, one possible mechanism of disrupted endothelial barrier integrity might be enhanced calcium-calmodulin dependent activation of myosin light chain kinases and Rho GTPases, which in turn phosphorylate cadherins and

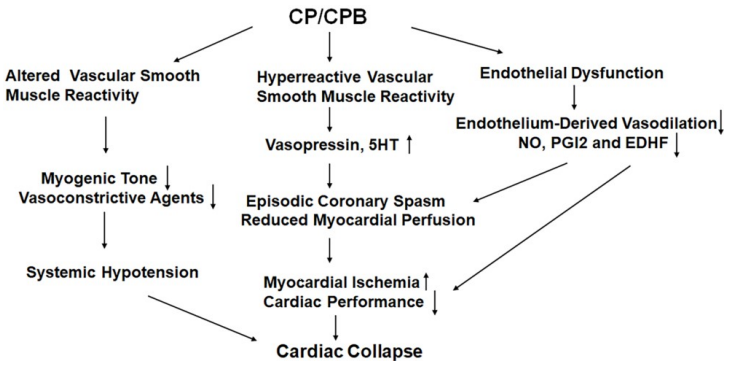

Figure 1. Schematic of general alterations in microvascular reactivity after cardioplegia (CP) and cardiopulmonary bypass (CPB). 5-HT: Serotonin; NO: nitric oxide; PGI2: prostaglandin I2; EDHF: endothelium-derived hyperpolarizing factor. Side arrows indicate overall direction of specific changes.

catenins, tagging them for degradation[118]. The angiopoietin-Tie2 ligand receptor system may also be implicated in endothelial barrier dysfunction following CPB. Angiopoietin 2 binding to Tie2 receptor increases the responsiveness of the vascular endothelium to pro-inflammatory cytokines, and levels of angiopoietin 2 have been shown to increase in serum following CPB[119].

Future considerations and directions

Among patients undergoing CP/CPB and cardiac surgery, further work needs to be done to examine the effects of other comorbidities on postoperative endothelial dysfunction. A good amount of work has been done in patients and animals with diabetes, with studies suggesting that declines in overall coronary and peripheral microvascular reactivity following CP/CPB are more pronounced in diabetic versus nondiabetic patients[31,116,120-122]. More specific differences between diabetic and nondiabetic patients after CP/CPB include enhanced upregulation of COX2 and enhanced inactivation of small and intermediate calcium-sensitive potassium channels[89,97,123]. Next, even though hypertension has been shown to cause significant changes to the microvasculature through mechanisms such as heightened reactive oxygen species production and perivascular inflammation, little work has been done to investigate specific contributions of pre-existing hypertension in vasomotor/endothelial dysfunction following CPB[124,125]. Similarly, the contributions of other preexisting conditions, such as hyperlipidemia, and other clinical variables such as age and sex deserve further investigation. Most previous studies on microvascular function were conducted in the immediate aftermath of CP/CPB and cardiac surgery. However, it is largely unknown how long the CP/CPB-driven endothelial and vasomotor changes persist. We hypothesize that many of these changes will last for several weeks post-CP/CPB; however, further studies are necessary to confirm any hypotheses.

CONCLUSION

Vascular changes that occur in most vascular beds during surgery that may lead to altered myogenic, agonist-induced and neurohumoral control of organ perfusion [Figure 1]. These microvascular and macrovascular changes occur due to inflammatory influence such as increased leukocytes and cytokines, increased oxidative stress, complement activation, and other factors such as receptor denaturation. Endothelium, vascular smooth muscle and extravascular cells may all be affected. These changes may affect organ perfusion and lead to myocardial infarction, stroke and renal failure. A better understanding of the effects of cardiac surgery on the vasomotor regulation and other forms of microvascular dysfunction after surgery may allow us to better care for patients after cardiac surgery and improve outcomes.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of this review article: Feng J, Kant S, Sellke FW

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Consent for publication

Not applicable.

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